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NUTRITION REVIEWS

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Nutrition: The Forgotten Art, by Daniel B. Stone 1

Clinical Nutrition

Use of Anabolic Hormones as Growth Stimulants 2
Zinc Metabolism in Laennec's Cirrhosis 4
Dietary Studies in Epidemiological Surveys 6

Experimental Nutrition

Xanthurenic Acid Excretion Resulting from Hydrazides 8
Effects of Meal Eating Versus Nibbling on Body Composition 9
Copper Toxicity in Kidneys 11
Influence of the Intestinal Flora on the Growth Rate of Mice 12
Irradiated Beef and Vitamin K 14
Absorption of Amino Acids 16
Fat Deficiency and Pituitary-Gonad Relationships 17
Vitamin E Deficiency in the Guinea Pig 20
Thiamine and Liver Lipids 21
Inhibition of Cortisol-Induced Gluconeogenesis by Pyridoxine Deficiency 22
Restriction of Food or Water and Work Output 23
Dietary Fats and Atherosclerosis in Chickens 25
Iron Enzymes in Iron Deficiency 27
Necrotizing Cardiopathy Associated with Chloride Deficiency 28
Copper Deficiency and Reproductive Failure 29

Notes

Letter to the Editor 31
American Board of Nutrition Announcement 31
Blood Serum Proteins of the Hippopotamus 31
Recent Books 32

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NUTRITION REVIEWS

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NUTRITION: THE FORGOTTEN ART

What is the most important problem in nutrition today? Let us examine some facts about a few illnesses of our time.

First, consider obesity. We know that persistent obesity impairs health and shortens life. Obesity favors cardiovascular renal disease, appendicitis, biliary calculi, liver and gall bladder cancer, puerperal complications, diabetes mellitus, tight collars and flat feet. We know also that man does not contradict the laws of thermodynamics. People get fat because they eat too much. How have these facts been put into practice? Recently, A. J. Stunkard has emphasized that weight-reduction programs are sometimes harmful and usually ineffective (Stunkard and M. McLaren-hume, *Arch. Int. Med.* **103,** *79* (1959). We all know the results. The cheat, the quack and the vitamin man have taken over. At a time when twenty million people gape at the television at any one moment, the quack has found a tremendous outlet for his propaganda. The fat patient finds himself in the center of turmoil and confusion.

Secondly, consider diabetes mellitus. The crucial problem in this illness is that vascular disease ultimately develops in most diabetic patients. Much evidence suggests that good control favors a longer and healthier life for the diabetic, whereas poor control encourages vascular disease. Consider how these facts have been put to use. Several experts have reported the accuracy of regulation in special groups of patients, patients who have been instructed, encouraged and studied for many years. Apart from selected groups, there is little information in the literature about the accuracy of diabetic regulation. R. E. Tunbridge studied the ability of 94 patients to maintain dietary control over a period of one week. Adherence to diet was accurate in 16, fair in 44, and hopeless in 34. He speculated that failure of dietary regulation might be due to psychological, social or educational causes (Tunbridge, *Lancet* **II,** *893 (1953)*). W. H. Daughaday wrote that it must be admitted that a high and largely undetermined percentage of the diabetic population fail to follow prescribed diets (Daughaday, *Nutrition Reviews* **17,** *289 (1959)*). One might guess that a disturbing number of diabetics are poorly regulated.

Thirdly, consider food faddism. The Food and Drug Administration has estimated that 50,000 salesmen carry food supplements from door to door. Food quackery is said to be a half billion dollar business that catches more than ten million American food faddists.

What generalizations can be drawn from these examples? I suggest that the pressing problem of nutrition today is less concrete, but no less important, than those of the past. The issue is one of emphasis. In all these examples we have acquired facts. In all three, the facts are not being used. Knowledge has outstripped practice. We have been eager to pursue facts, but slow in transmitting them. This disparity does not apply to nutrition alone. It is one of the philosophical problems of our time. We are living in an age in which we have made vast stores of riches but have not learned to use them wisely.

Most physicians know much about obesity, diabetes, and food faddism. Are they teaching their patients? The question is not whether physicians have done well, or whether they are doing better than they have done in the past, but whether they are meeting the challenges and opportunities of the times. Specialization has created huge stores of facts and a big demand for teachers. We need people with intellectual and technical competence to translate and teach.

Research and teaching are like the wheels of a bicycle. Both are essential. Both should be going the same way at the same speed. At present, research is the big wheel. I suggest, not that we put the brake on this, but that we increase the size and speed of the teaching wheel. Our main business is not only to see what lies dimly at a distance but also to do what lies clearly at hand.

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USE OF ANABOLIC HORMONES AS GROWTH STIMULANTS

A controlled study of prematurely born infants revealed no effect on growth from the use of 17-alpha-ethyl-hydroxynorandrostenone. This drug is known to produce hepatic dysfunction in adults.

In recent years there has been a continuing search for substances, both dietary and medicinal, which would accelerate the growth of infants and children. Favored among the subjects for study has been the prematurely born human infant. Under normal circumstances such infants actually lose weight for the first few days of life, and it is not until one to three weeks have passed that growth in weight begins. Usually this period of absent or very slow growth is longer the smaller the infant (*cf.* chart in Nelson's *Textbook of Pediatrics, 7th ed., p. 311, W. B. Saunders Co. (1959)*). Once growth begins, the healthy premature infant will grow at a rate which is not far removed from the projected intra-uterine growth rate. However, repeated attempts to stimulate the growth of such infants have been made, particularly in those infants whose growth rate is judged to be subnormal.

Recently, a new anabolic steroid, 17-alpha-ethyl-hydroxynorandrostenone, a testosterone-like compound, has become available for human use. This steroid is reported to have an anabolic effect equal to that of testosterone, with only one sixth of its androgenic effect. Norethandrolone, as it shall hereafter be referred to, has been found to promote weight gain in undernourished adults (R. N. Watson *et al.*, *Am. J. Med.* **26,** *238 (1959)*). Moreover, testosterone itself has been shown to promote nitrogen retention in children, even in the face of a restricted calorie intake (*Nutrition Reviews* **4,** *289 (1946)*; *Ibid.* **6,** *179 (1948)*).

It is in this setting that R. W. Meadows *et al.* (*Am. J. Dis. Child* **99,** *206 (1960)*) undertook a controlled study on the effects of norethandrolone on the growth of prematurely born infants. A total of 21 Negro infants (10 male and 11 females) weighing between 900 and 1660 g. at birth were available for study. The infants were assigned in rotation to three groups: the first receiving no hormone, the second an oral dose of 1 mg. norethandrolone per kg. of body weight per day, and the third a dose of 2 mg. per kg. of body weight. Administration was begun in each instance on the third day of life, and continued until a body weight of 2300 g. was attained. On the average, this weight was reached in 48 days (range 33 to 85 days). The infants were observed for an additional three to five days before they were discharged from the nursery.

In all other respects, the infants received the same care. Nothing was given by mouth for the first 36 hours of life. Feedings began with glucose-water, and gradually progressed through dilute cow's milk mixtures to a standard formula by the third week of life. This formula provided 145 calories, 6.0 g. of protein, and 200 ml. of fluid per kg. of body weight per day. Thus the infants were fed according to a prescribed schedule based on body weight and were not allowed to feed on demand.

The infants were carefully observed, and frequent measurements were made of body weight, body length, total serum protein, serum bilirubin, blood non-protein nitrogen, and serum cholesterol. Weekly hematological studies included hemoglobin, hematocrit, erythrocyte, leukocyte, and reticulocyte counts, and leukocyte differential counts. Roentgenograms were taken of some of the infants in order to evaluate the degree of skeletal maturation.

Comparison of the treated and untreated infants on the basis of these criteria revealed no significant differences which could be ascribed to the hormone administration. The control infants gained an average of 19 g. per day for the entire period of observation, those getting 1 mg. of norethandrolone 20 g. per day, and those getting twice this dose, 21 g. per day. Gain in length averaged 0.12, 0.14, and 0.14 cm. per day for the three groups, respectively. The authors state that in each instance these differences are not statistically significant. Upon discontinuing the hormone no instance of weight loss or failure to gain was observed.

From the standpoint of the blood chemical and hematological data the three groups of infants were comparable in every respect. No differences in degree of skeletal maturation were found. No clinical evidence of androgenic effect could be detected in the treated groups.

In essence, the authors failed to detect any evidence that norethandrolone will promote growth of prematurely born human infants. In evaluating this study, however, note should be taken of the fact that the feedings offered to the infants contained large amounts of protein; the amount consumed was 6.0 g. per kg. of body weight per day. It is known that prematurely born infants will thrive on formulas providing one half this amount of protein. Under the circumstances of this study, the infants may well have been storing protein at a maximum rate, so that the addition of a growth "stimulant", regardless of type, could not be expected to show results.

No mention is made in the article of vitamin supplements to the diet. The milk preparation which forms the basis of the formula used is fortified with vitamins C and and D, but the quantity of milk offered to the infants would not supply these supplements in the amounts which are considered adequate. It would be important to know whether the authors actually omitted vitamin supplements, or merely failed to include mention of these in the manuscript.

Of the utmost importance to the clinician is the matter of potential toxic effects from the use of norethandrolone. In the premature infant study, no toxic effects were observed. But a recent report by B. S. Gordon *et al.* (*Am. J. Clin. Path.* **33,** *156 (1960)*) indicates the potential hazard of this drug.

These authors report two adults who died of liver failure following prolonged administration of norethandrolone. The first patient, a 43-year-old male, had pemphigus vulgaris for which he received corticosteroid therapy. Complicating severe osteoporosis was the indication for norethandrolone therapy, the drug being given in a dose of 30 mg. per day for a period of about six months. Clinical evidence of liver dysfunction was noted in the fifth month of treatment, and the patient died in hepatic coma.

The second patient, a 57-year-old female, was given norethandrolone (20 mg. daily) and corticosteriods in an attempt to treat a chronic pancreatitis of long standing. Evidences of hepatic dysfunction appeared during the tenth month of therapy, and she, too, died in hepatic coma.

In each instance the microscopic examination of the liver revealed a striking appearance. There were large "lakes" of blood scattered throughout, most of which were lined by sinusoidal epithelium. Occasionally there was evidence of thrombosis, and some of the "lakes" were connected with the lumen of a vein. Presumably these are the result of necrosis of liver cells, although toxic damage to blood vessel walls is also a possibility. This condition, the exact pathogenesis of which is not clear, is known as "peliosis

hepatitis." The other significant finding in each case was a severe degree of cholestasis. The intralobular and perilobular ducts contained inspissated bile, and clumps of bile could be seen in the Kupffer cells.

In the first patient there was a moderate degree of necrosis of the cells in the central portion of the liver lobules, but no inflammation of the portal areas. However, the liver of the second patient, in addition to peliosis and cholestasis, showed evidence of a severe viral hepatis.

The histological picture here is similar to, but much more severe than, that which has been seen in liver biopsy specimens from a number of adult patients treated with norethandrolone (F. Schaffner, H. Popper and F. Chesrow, *Am. J. Med.* **26,** *249 (1959)*). Many also show biochemical evidence of hepatic dysfunction, such as bromsulphthalein retention, and elevation of serum bilirubin, alkaline phosphatase, and glutamic-oxalacetic transaminase. However, both the biochemical and histological abnormalites appear to be reversible on discontinuing the hormone.

In the two fatal cases reported by Gordon *et al.*, no other drugs had been given which could have caused the striking hepatic abnormalities. Obviously, each patient suffered from a chronic debilitating disease, the second had a complicating viral hepatitis, and the authors properly refrain from making a judgement as to whether the norethandrolone therapy was directly responsible for their deaths.

In the premature infant study neither liver biopsies nor function tests, aside from serum bilirubin determinations, were undertaken. However, no clinical evidence of liver malfunction or damage was found. The premature infant may possibly tolerate norethandrolone better than the adult. But in view of the reports of definite toxicity in the older age group, it would seem unwise to prescribe this hormone without careful observation of the status of liver function. Certainly, norethandrolone does not accelerate the growth rate of the healthy premature infant.

ZINC METABOLISM IN LAENNEC'S CIRRHOSIS

Zinc deficiency, conditional on the presence of the underlying disease and remedied by supplements of zinc taken orally, is described in patients with postalcoholic cirrhosis.

Data suggesting a conditioned deficiency of zinc in patients with postalcoholic cirrhosis has been collected by B. L. Vallee, W. E. C. Wacker, A. F. Bartholomay and F. L. Hoch (*Ann. Int. Med.* **50,** *1077 (1959)*).

Studies of zinc concentration were made in serum from 40 normal controls and 33 patients with Laennec's cirrhosis; in urine, from 14 normal controls and nine patients; in samples of liver obtained at autopsy, from seven patients without liver disease and from five patients with histologically confirmed cirrhosis. Methodology has previously been described (Hoch and Vallee, *J. Biol. Chem.* **181,** *295 (1949)*; J. H. R. Kaegi and Vallee, *Schweiz. med. Wchnschr.* **88,** *136 (1958)*; Vallee, *Principles of Quantitative Biological Emission Spectography, Symposium on Spectrochemical Analysis for Trace Elements, Special Technical Publication No. 221, Am. Soc. for Testing Materials, Philadelphia, (1958)*; Vallee and J. G. Gibson, II, *J. Biol. Chem.* **176,** *435 (1948)*).

Bromsulfalein retention was measured as an index of liver function. In six of the patients, the serum and urine zinc concentrations and the retention of bromsulfalein were studied before and during the oral administration of zinc sulfate (one 30 mg. capsule) three times daily, an amount equal to a total of 19.5 mg. of zinc daily, which is somewhat greater than the normal human dietary intake of 10 to 15 mg. daily.

The diet per se of these patients contained an estimated 15 mg. zinc per day.

The data provide evidence for a decreased serum zinc concentration in patients with Laennec's cirrhosis. The mean serum concentration per 100 ml. of serum for the normal control group was 120 ± 19 μg., while the mean concentrations per 100 ml. of serum for the cirrhotic group were recorded as follows: severe disease, 66 ± 19 μg.; mild disease [by previously described criteria (Vallee, Wacker, Bartholomay and E. D. Robin, *New Engl. J. Med.* **255,** *403 (1956)*)], 87.8 ± 20 μg. The data show, in addition, an increased excretion of zinc in patients with Laennec's cirrhosis.

The urine zinc content of the normal control group was 457 ± 120 μg. per 24 hours; of the group with cirrhosis, 1030 ± 188 μg. per 24 hours. The data also demonstrate a decrease in zinc concentration in liver obtained at autopsy, whether expressed on a basis of wet weight, dry weight or nitrogen content. For example, the livers from patients dying from nonhepatic disease had a mean zinc concentration per gram of wet weight of 69 ± 23 μg., whereas the livers of patients dying with cirrhosis had a mean zinc concentration of 29 ± 8 μg. Bromsulfalein retention was directly correlated to the severity of disease and the extent of the disorder of zinc metabolism.

Supplementation of the diet of patients with postalcoholic cirrhosis with physiological quantities of zinc resulted in the disappearance of zincuria. Doubling the dose, however, (*i.e.*, the administration of 39 mg. of zinc daily), resulted in a recurrence of zincuria in the one patient tested. The authors point to the consistent decrease in bromsulfalein retention in all six patients who received additional zinc. Four of the six patients showed a slight increase in serum zinc concentrations. The lack of any evidence of spontaneous recovery in these patients prior to zinc supplementation adds to the significance of the observations.

The correlation between an abnormal zinc metabolism and increased bromsulfalein retention seems to be more apparent in Laennec's cirrhosis than in other diseases of the liver. In eight patients with infectious hepatitis, for example, the authors found a mean zinc concentration per 100 ml. of serum of 107 ± 25.4 μg. compared to the normal control value of 120 ± 19 μg.

The importance of these observations lies in the fact that zinc has not previously been regarded as important in the pathologic biochemistry of postalcoholic cirrhosis and that focusing on these abnormalities provides a new and different approach to the disease. The authors embarked on these clinical studies following discovery of zinc metalloenzymes in liver tissue (D. Keilin and T. Mann, *Biochem. J.* **34,** *1163 (1940)*; Vallee and Hoch, *J. Biol. Chem.* **225,** *185 (1957)*). In addition, they noted with interest the use in India of zinc in the therapy of infantile hepatic necrosis (M. B. Prabhu, *Indian J. Pediat.* **7,** *122 (1940)*), a disease which may have, in part, a nutritional basis.

The authors explain the abnormalities observed in the present studies as being caused by a zinc deficiency, which is most likely a conditioned deficiency in which the normal zinc intake does not meet the patients' needs because of the presence of unusual secondary factors related to the underlying disease (M. G. Wohl and R. S. Goodhart, Editors, *Modern Nutrition in Health and Disease, 2nd Edition. Lea and Febiger, Philadelphia, (1959)*). They point to the direct relationship between the high calcium intake from forage and the severity of porcine parakeratosis (an endemic zinc deficiency disease) as an example of a conditioned deficiency of zinc (P. K. Lewis, Jr., W. G. Hoekstra, R. H. Grummer and P. H. Phillips, *J. Animal Sci.* **15,** *741 (1956)*).

Zinc deficiency, whatever its cause, creates a biochemical defect in all probability at the sites of the metalloenzymes. The zinc metalloenzymes participate in the dehydrogenation of ethanol (Vallee and Hoch, *loc. cit.*) and glutamic acid (Vallee, S. J. Adelstein and J. A. Olson, *J. Am. Chem. Soc.* **77,** *5196*

(*1955*)), two substrates involved in the metabolic changes accompanying cirrhosis. Numerous transphylar analogies suggest areas of metabolism possibly affected by defects in zinc metabolism in man, *e.g.*, protein balance and alcohol detoxication. Mammalian alcohol dehydrogenase has, unlike its counterpart in yeast, gained a capacity to oxidize other alcohols, including vitamin A_1 (A. F. Bliss, *Arch. Biochem. Biophys.* **31,** *197* (*1951*)). In fact, vitamin A_1 alcohol dehydrogenase and liver alcohol dehydrogenase are apparently the same enzyme, containing 0.18 per cent zinc (Bliss, *loc. cit.*; Vallee and Hoch, *J. Biol. Chem.* **225,** *185* (*1957*)). Hence, studies of A. J. Patek, Jr., and C. Haig (*J. Clin. Invest.* **18,** *609* (*1939*)) demonstrating a defect in dark adaptation resistant to vitamin A therapy in alcoholic cirrhosis may have reflected altered zinc metabolism in this disease.

The authors present some new thoughts on an old and difficult problem. While their arguments are generally convincing, they have not discussed the important possibility that a primary cause of the low serum zinc concentrations, the zincuria and the low tissue zinc concentrations in cirrhosis, may lie in an alteration in the clearance of zinc by the kidney. This possibility suggests further physiologic investigations of the renal handling of zinc in normal and cirrhotic man together with biochemical studies of renal as well as hepatic tissue.

DIETARY STUDIES IN EPIDEMIOLOGICAL SURVEYS

Present methods of dietary surveys are discussed and suggestions for increasing reliability, validity and simplicity are advanced.

The potentially great value of dietary studies in epidemiological surveys for uncovering possible relationships between the dietary habits and diseases of man suffers from several inherent weaknesses. For example, a truly accurate analysis of the diet of an adequate number of individuals is so time consuming that it is generally not practicable. On the other hand, appraisal of diet by questioning of individuals or by use of tables of food values may produce results so inaccurate as to be practically useless. Three papers appearing in the *American Journal of Public Health* have analyzed the present status of such surveys and have suggested improvements in presently used systems.

C. M. Young and M. F. Trulson (*Am. J. Pub. Health* **50,** *803* (*1960*)) have surveyed current methods of dietary evaluation. They point out that it is considerably simpler and less expensive to obtain information on the mean intake of a group than to evaluate the dietary habits of the individual. Nevertheless, the greater information obtained by the latter method may compensate for the additional difficulty.

Questions which must be settled before any survey can be initiated include: the time span to be studied (the shorter times give greater ease and accuracy but may miss important trends); the type of information desired (calories, essential nutrients, daily and seasonal patterns, and so forth); and the methods of collection of data (by interview, questionnaire, and the like). In each case the desired degree of accuracy and reliability that is obtainable by the method used must be considered. This latter consideration is of great importance as shown by results of past dietary surveys in which different methods of obtaining information have produced very different results. It was apparent that most subjects could not recall how much they ate with any degree of accuracy.

It is probably for this reason, as well as the great cost of conducting surveys with trained interviewers and large numbers of subjects, that most of the current studies are short-term and limited in their objectives.

However, it has been found that a one- or two-day study, though certainly more accurate than a longer-term survey by recall and much less expensive than long-term accurate measurement of food intake, may be quite misleading because of daily variation of diet. Even two weeks may not be a sufficient period because of differences in week-day and week-end diets; even in this length of time, inaccuracy of recall may invalidate the data.

The conclusion reached by the authors is that because of the doubtful validity of current methods of dietary surveys it is difficult to evaluate their usefulness. It is evident that new methods are needed which incorporate some system of testing their validity. It is also possible that studies comparing the dietary habits of individual members of a group with the mean may reveal that in many cases the more simple, less accurate means will serve a useful purpose.

In a further discussion of the accuracy of current methods, M. G. Whiting and R. M. Leverton (*Am. J. Pub. Health* **50,** *815 (1960)*) have compared results of collecting and processing dietary data by two methods: laboratory analysis and calculation from food-value tables. For collection of data, it was evident that precise weighing of food actually eaten is by far the most accurate method. Laboratory analysis of representative samples of the weighed food then reveals, with considerable accuracy, the content of the various nutrients. However, the method is expensive and time consuming and thus does not lend itself to long-term studies with many subjects.

For the food tables, it should be emphasized that values reported are averages and are accurate only to the extent that they represent many determinations from different locations at different times. Perhaps the greatest source of inaccuracy, however, is the difficulty of accounting for all the ingredients of mixed dishes, especially if not prepared at home. It is apparent that if truly reliable results are to be obtained from food tables, common or usual foods, simply prepared, must be used, descriptions must be accurate and adequate times of study and numbers of individuals must be used.

A comparison of the two methods of processing diet information was made from approximately 300 cases recorded in the literature. It was found that, for protein and calories, over 50 per cent of the values calculated from the tables fell within a range of 10 per cent of the analyzed values. For calories, 32 per cent of the calculated values were more than 10 per cent above the analytical values while, for protein, 30 per cent were more than 10 per cent below the analytical values. However, 49 per cent of the calculated values for fat were more than 10 per cent above the analytical values. The discrepancy was increasingly great for the higher fat levels (greater than 35 g. per day). Some of these differences may be due to the fact that most tables contain values for fat calculated from carcass or wholesale cuts of meat, whereas much of the fat is lost by trimming or cooking before consumption.

These studies emphasize the necessity, considered above, for frequent testing of shorter simpler methods of dietary analysis against the more detailed accurate methods so that some confidence can be placed in the former.

As a partial solution to some of these problems, D. G. Wiehl and R. Reed (*Am. J. Pub. Health* **50,** *824 (1960)*) have considered the development of new and improved methods of obtaining dietary information. The authors point out that although very accurate studies of specific nutrients may produce valuable results, there is a need for a short simple method which will reveal differences in general overall dietary patterns of large populations.

The short questionnaire for this study should be distinguished by two features: first, the questions should be simple but clear enough to be used by nontechnical field personnel or self-administered; second,

they should be so carefully planned that, even though accurate dietary analysis would not be possible, at least broad differences could be easily recognized. The choice of questions would naturally be dictated by the type of answers required and would be applicable only to the study under consideration. They should also be formulated in such a manner that the answers could be easily tabulated and would readily reveal any differences. Reliability would be tested by a second questioning of the same population, and validity by accurate testing of the diets of a sample of the population. Any differences in dietary habits revealed by the short questionnaire could then be checked by a more detailed analysis.

The authors suggest a few of the types of questions which would be revealing. One series of questions could be directed toward determining whether the usual diet was high in fat, protein or carbohydrate. A second could involve frequency of eating, especially whether there was considerable between-meal eating. Questions concerning alterations in dietary habits and weight changes would be important.

It is evident that considerable time and effort should be expended to make certain that the questions asked were the best for the purpose. However, once this was accomplished, the remainder of the survey might be considerably simplified and more accurate.

XANTHURENIC ACID EXCRETION RESULTING FROM HYDRAZIDES

Desoxypyridoxine and several hydrazides produced convulsions in dogs. The hydrazides produced xanthurenuria. Pyridoxine reversed these effects promptly.

Six years ago J. P. Biehl and R. W. Vilter (*J. Am. Med. Assn.* **156,** *1549 (1954)*) compared the peripheral neuritis which developed in tuberculous patients receiving isoniazide to that observed by them in pyridoxine deficiency. They found that large doses of this hydrazide resulted in an increase in the urinary excretion of pyridoxine. Furthermore, administration of pyridoxine prevented the development of the neuropathy which otherwise occurred in approximately 40 per cent of their patients. The patients excreted abnormal quantities of xanthurenic acid after being given a loading dose of tryptophan, but pyridoxine reversed this abnormality also.

J. F. Mueller and Vilter (*J. Clin. Invest.* **29,** *193 (1950)*) reported the effects of administration of desoxypyridoxine to patients and observed that they developed an illness characterized by scaling of the skin, gastrointestinal disturbances and abnormal rates of urinary excretion of xanthurenic acid after administration of tryptophan. These abnormalities were reversed when pyridoxine was given.

Quantitative studies of the urinary metabolites of tryptophan were conducted by J. M. Price, R. R. Brown and F. C. Larson (*J. Clin. Invest.* **36,** *1600 (1957)*), who gave isoniazide or desoxypyridoxine to human subjects. Following the administration of either of these drugs, the subjects, in response to tryptophan loading, excreted several metabolic derivatives of this amino acid. Those who were given desoxypyridoxine were found to excrete in quantitative order, hydroxykynurenine, kynurenine and xanthurenic acid. Subjects who had been given isoniazide excreted kynurenine, acetylkynurenine and xanthurenic acid.

Normally, tryptophan is converted to kynurenine, which is metabolized. Pyridoxine deficiency may result in incomplete metabolism of this substance, resulting in abnormal excretion of it or of its derivatives. The fact that different pyridoxine antagonists result in slightly different excretion products might be interpreted as evidence

that the metabolic block occurs in different portions of the pathway.

Many of the hydrazides and desoxypyridoxine have a convulsant effect. H. L. Williams and R. G. Wiegand (*J. Pharmacol. Exp. Therap.* **128,** *344 (1960)*) studied the acute effects of several of these compounds on the rate of urinary excretion of xanthurenic acid by dogs given an infusion of tryptophan. The authors found that four of these (thiocarbohydrazide, thiosemicarbazide, semicarbazide, and isonicotinoyl hydrazide) produced prompt increases in the rate of xanthurenic acid excretion, but desoxypyridoxine did not manifest this acute effect. Nevertheless, all of these substances proved to be potent convulsive agents. The relative potency of the hydrazides in producing xanthurenuria correlated roughly with their convulsant potency (but desoxypyridoxine was the exception).

The authors considered that these drugs produced an acute transitory deficiency of pyridoxine since administration of the vitamin abolished both xanthurenuria and convulsions almost immediately.

The differences between the actions of desoxypyridoxine and of the hydrazides becomes apparent through this study. The former drug, although a convulsant, did not produce xanthurenuria in a matter of minutes, whereas the hydrazides did. Pyridoxine antagonized the convulsant effect of all of these drugs equally well. Although a deficiency of pyridoxine has been known to produce convulsions in young infants (S. E. Snyderman *et al., J. Clin. Nutrition* **1,** *200 (1953)*; C. J. Molony and A. H. Parmelee, *J. Am. Med. Assn.* **154,** *405 (1954)*; D. B. Coursin, *Ibid.* **154,** *406 (1954)*), trials of pyridoxine in patients with Parkinsonism failed to modify this disorder (W. H. Barker *et al., Bull. Johns Hopkins Hosp.* **69,** *266 (1941)*), a finding which would suggest that the vitamin itself does not have an anticonvulsant pharmacologic action.

This study presents certain important facets of the relationship between pyridoxine, tryptophan metabolism, and a broad group of convulsant drugs which seem to exert an anti-pyridoxine effect. The fact that an immediate response occurs upon administration of these drugs is quite unusual and should elicit further interest in the actions of antimetabolites.

EFFECTS OF MEAL EATING VERSUS NIBBLING ON BODY COMPOSITION

When rats were force fed their total daily intake as two meals they deposited more fat and less protein than control animals allowed to nibble throughout the period.

In addition to the effect of feeding individual nutrients upon the growth and welfare of animals and man, data has appeared showing that certain nutrients are more beneficial when fed together or within a short period of time. The rate at which mixtures of amino acids are fed has been shown to be important (*Nutrition Reviews* **17,** *87 (1958)*). If an animal does not get all of the essential amino acids at one time he cannot store the incomplete mixture until adequate amounts of the missing substances appear.

In many nutritional studies when one group of animals does not eat as well as another, the latter group is force fed. Thus, as long as comparable amounts of nutrients are consumed by the animal, no further interest is paid as to whether the daily intake is given in multiple doses or as a single meal.

Recently, C. Cohn and D. Joseph (*Metabolism* **9,** *492 (1960)*; *Nutrition Reviews* **18,** *334 (1960)*) reported studies in which the effects of feeding rats their entire daily food intake in two large meals were compared to the ad libitum feeding. Under normal conditions the rat frequently eats small

amounts of food throughout the day; these investigators designated this type of feeding "nibbling." The animals were compared on a pair-gain basis so that both groups gained similar amounts (63 *vs.* 64 g.). It was found that the bodies of animals allowed to eat ad libitum contained 20.4 g. of fat (9.6 per cent of the body weight), while the bodies of force-fed animals contained 39.0 g. of fat (17.6 per cent of the body weight). Moreover, the rats fed ad libitum gained only 0.3 g. of fat per animal while the force-fed animals acquired 23.7 g. of fat during the two-week study period.

In a second series of studies, two groups of eight rats each were either force fed their daily intake in two divided doses or allowed to eat ad libitum. Since the pair-feeding technique was employed, both groups of animals had approximately the same intake. After a two-week period, the rats fed ad libitum (nibblers) gained an averaged of 65 g. and those force fed (on the meal-eating schedule) gained 62 g. Since there was no indication of the degree of spread around these means, it is to be assumed that the weight difference was not significant.

The nibblers gained an average of 12.9 g. of protein each, while those force fed gained only 9.9 g. of protein. By contrast, the ad libitum-fed rats gained only 4.9 g. of fat per animal while the force-fed animals gained an average of 13.2 g. of fat.

Thus, it appears that the manner in which the diet is consumed, either as nibbling or in the form of large meals, can induce alterations in the general body composition of the rats. When a natural nibbler is made to take his food in the form of large meals, he conserves more of the calories and stores them in the form of fat.

When determining the efficiency of a diet, the amount of weight gained per unit weight of food consumed is compared. Under such conditions, it is assumed that body composition does not change with respect to the fat, protein and water content. In the present experiment, according to this classical technique, no difference in efficiency of food utilization would be found between feeding methods since the weight gain for both groups was similar. However, if one calculates the ratio of calories retained to calories consumed using the standard caloric values for fat and protein (the carbohydrate content of the animals was disregarded), this ratio would be 0.206 for the force-fed animals and only 0.119 for the animals eating ad libitum.

Thus, on this basis of comparison, it appears that the diet was about twice as efficient when consumed as a meal than when eaten ad libitum.

In an effort to explain why the body composition of rats is altered by changing eating habits, these investigators studied some of the intermediary pathways necessary for fat formation. It is known that reduced triphosphopyridine nucleotide (TPNH) is necessary for reductive steps in fatty acid biosynthesis. This reduced coenzyme can be generated through the oxidation of hexosemonophosphate and phosphogluconate, steps in the hexosemonophosphate shunt mechanism of glucose metabolism.

To evaluate this pathway of metabolism the authors carried out two types of measurements: (1) the combined activity of the two enzymes which generate TPNH was measured in the supernatant fluid from homogenates of liver and epididymal fat pads; and (2) the conversion of C-1 and of C-6 labelled glucose to labelled carbon dioxide was measured in liver slices. The latter method has been used extensively to estimate the relative activity of the hexosemonophosphate shunt, an increased ratio of labelled carbon dioxide from C-1 over C-6 indicating increased shunt activity. Although this type of measurement is open to some criticism, it should have some validity when making comparative measurements between two types of animals. The data presented on the tissues of rats on a high carbohydrate diet, force fed versus ad libitum, support the conclusion of an increased activity of the hexosemonophosphate shunt in the force-fed animals, which should favor lipogenesis.

Thus, the study by Cohn and Joseph tends to substantiate their concept that there is a

difference in body composition between rats fed ad libitum and those fed equivalent amounts of food but ingesting large quantities at one time. Although this factor heretofore has not been stressed by nutritionists, it may be important in not only animal studies but as a causative agent in various disease states now becoming more prevalent in man. Man, because of social conditions, has become a meal eater rather than a nibbler, except in his infancy and early childhood.

At present, the mechanism causing these differences in composition is not understood and, for this, considerable investigation will be required. Consideration of meal eating versus nibbling may be important in attempts to produce in experimental animals (particularly the rat) diseases and metabolic abnormalities occurring in man.

COPPER TOXICITY IN KIDNEYS

Ionized copper combined with bovine albumin was injected into mice in order to localize the site at which deposition of copper occurs and to get information about the resulting renal pathological changes.

Persons suffering from hepatolenticular degeneration or Wilson's disease are deficient in a copper-binding globulin known as ceruloplasmin. As a result, copper is probably transported as a loose complex with albumin, is deposited in many tissues including the kidneys, and is excreted in excessive quantity (hypercupruria). The fact that hepatolenticular degeneration is frequently characterized by aminoaciduria, glycosuria, proteinuria, calciuria and phosphaturia suggests the possibility that copper is the responsible nephrotoxic agent producing these abnormalities.

The manner in which copper travels through human kidneys in unknown and the extent of its cellular physiologic effect is obscure. The study of F. S. Vogel (*Am. J. Path.* **36,** *699 (1960)*) was directed toward information about the role of copper in the pathogenesis of the renal abnormalities of Wilson's disease. Young adult mice of the AKD_2/JAX strain weighing 20 to 30 g. were used.

The copper-albumin complex was prepared by immersing the albumin solution contained in cellulose tubing in a dilute solution of copper sulfate for six hours followed by a three-day dialysis in running tap water.

Forty mice were injected intraperitoneally with a solution of copper-albumin complex containing 366 μg. of copper per animal. A similar volume of bovine albumin solution was injected into each of 24 control mice. At intervals from one to 144 hours later pairs or groups of four animals were sacrificed. A group of 20 mice was given a similar injection of copper-albumin three days after double ligation of the left ureters just distal to the renal pelvis.

The effects of repeated injections were studied in another group of mice. Animals were sacrificed at intervals after injections spaced five days apart.

The copper content of tissues was determined spectrophotometrically. In addition histologic and histochemical procedures were used to study the cellular alterations resulting from copper deposition.

Three to six times the normal amount of copper was deposited in the renal parenchyma within one hour after the injection and persisted for more than six hours. At this time the concentration decreased sharply and by 48 hours it was again normal. Liver and spleen levels of copper were high and remained elevated for a long time and the brain content of copper was also increased above normal.

Grossly the kidneys appeared normal for the first five hours after the injection. In the next two hours the organs enlarged and the cortex became congested. The maximum weight of 300 mg. (normal, 200 mg.) was ob-

served in animals sacrificed at 24 and 48 hours. The border between the congested medulla and blanched cortex was sharply outlined. Death was most common during this interval. Animals that survived for 72 hours had grossly normal appearing kidneys.

Rubeanic acid histochemical staining of renal tissues revealed the presence of an abundance of fine particles in the glomerular tufts and subcapsular spaces six hours after the injection of copper-albumin. After 24 hours more marked staining was present in the epithelium in both proximal and distal tubules. The fine granules were not found in nuclei but were irregularly distributed in the cytoplasm. The correlation between the amounts of copper determined chemically and stained histochemically was poor.

Tissues taken six hours after injection showed cytologic lesions. Vacuolation, pale staining and swelling of cytoplasm characterized the epithelial cells of the proximal convoluted tubule segment. Coagulative necrosis was evident frequently by 24 hours. The lesions of the tubules were severe including lysis, hypereosinophilia of the cytoplasm, pyknosis, karyorrhexis of nuclei and cellular desquamation lesions, but they were confined to the proximal convoluted tubules. Tubules contained cellular debris including granular basophilic material staining positively for calcium and phosphate but not for iron. Many basement membranes were ruptured and some erythrocytes were present in tubules. Renal parenchyma examined 72 to 144 hours after injection of copper-albumin showed predominantly epithelial regeneration, but there was also some residual evidence of degeneration.

In dissected nephrons the proximal convoluted segments of the tubules were dilated and the sloughed epithelium obliterated many of the nephrons examined. Amorphous, coarsely granular masses of cellular debris replaced the epithelium. The disruption of epithelium and tubular dilatation did not progress beyond the thin loop of Henle. Distal tubules appeared more normal except for the presence of numerous casts.

Ligation of the left ureter resulted in a slight increase in copper content of the left kidney but much greater increases were found in the opposite organ. Ligation of the ureter resulted in dilatation of tubules and atrophy of the epithelium but injection of copper-albumin did not accentuate these cell changes. Necrosis did not occur in kidneys with ligated ureters but rather in the opposite kidney.

Repeated injections of copper-albumin complex did not appear to intensify the epithelial necrosis seen in the proximal convoluted tubules after a single injection. An interval of 72 to 96 hours after the third injection was sufficient to permit virtually complete reconstitution of renal epithelium. Calcium deposition increased after several injections.

Not all animals survived the changes occurring as a result of copper-albumin injection. The results of these experiments indicate the possible hazard involved in trying to increase the excretion of copper by the kidney. The fact that the site of deposition of copper in the kidney corresponds to the region where the most severe cell damage occurs implicates copper itself as an important factor in the pathogenesis of the renal alterations occurring in Wilson's disease.

INFLUENCE OF THE INTESTINAL FLORA ON THE GROWTH RATE OF MICE

Mice raised under conditions so that they were free of specific microorganisms present in stock mice grew better on all the diets studied than did the stock animals.

With the advent of the insoluble sulfadrugs and later the use of certain antibiotics, it was possible to modify profoundly the intestinal flora of experimental animals

and man. Evidence has accumulated that the intestinal microorganism, rather than just playing a passive role, may be the supplier of many essential nutrients to the host (*Nutrition Reviews* **9**, *197* (*1951*); **16**, *305* (*1958*)).

At the Rockefeller Institute, a colony of Swiss mice has been developed which is free of specific pathogens (not germ-free). The colony originated from 12 mice obtained by Caesarian section from three pregnant Swiss mice. The young were nursed by foster mothers of the Princeton strain known to be free of pleuropneumonia-like organisms and enzootic bronchiectasis. These 12 mice, seven males and five females, were the breeding stock for the new colony, which was freed of intestinal parasites and ectoparasites by employing adequate therapy. The colony was maintained under conditions which would prevent contact with outside sources of infection (see J. B. Nelson, *Proc. Animal Care Panel* **7**, *30* (*1957*)).

Recently, R. J. Dubos and R. W. Schaedler (*J. Exp. Med.* **111**, *407* (*1960*)) have described nutritional studies employing mice from this colony. These mice have been designated as the NCS (Nelson-Collins-Swiss) strain while the albino mice raised under ordinary laboratory conditions were called the SS (Standard Swiss) mice, the group from which the NCS strain originated.

During the nutritional studies the animals were housed in individual cages and, for each series, seven SS mice (controls) and seven NCS (test) mice were each given ad libitum one of the following rations: standard mouse pellets, 15 per cent casein diet, 15 per cent gluten diet or 15 per cent gluten diet fortified with lysine. The animals were maintained on each of the given diets for three weeks and weighed weekly.

Animals fed the commercial mouse food gained the following average amounts by the third week of study: SS mice 5.4 g. and NCS mice 10.3 g. The SS mice on the 15 per cent casein diet gained 4.8 g. while the NCS animals eating the same diet had an average increase in weight of 9.0 g. The 15 per cent gluten diet with the low content of lysine permitted only a small weight increase by the SS mice (1.5 g.) while the NCS mice on the lysine-deficient diet had a good growth (7.9 g.). It should also be noted that the NCS mice on a poor diet had a better growth rate than the SS mice on the commercial mouse diet which was nutritionally adequate. Supplementing the 15 per cent gluten diet with lysine allowed the SS mice to gain an average of 3.9 g. while the NCS mice on this diet gained 10.5 g. over the three-week test period.

This study is interesting in that the mice that were free of specific pathogens (the NCS group) showed better growth on each of the four diets than did the control mice. Thus one may conclude that, if the growth rate of mice is taken as the index of the nutritional quality of a diet, one must also take into consideration the status of the animals' intestinal flora.

These investigators studied the intestinal flora of both groups of mice with respect to the type and number of microorganisms present. The most prominent microorganism in the intestine and feces of the NCS mice (as determined by ordinary culture media) were the lactobacilli and enterococci. There were also smaller numbers of unidentified Gram-negative bacilli. NCS animals protected from contamination did not have any organisms of the coliform group (lactose fermenters), and organisms of the paracolon group (slow lactose fermenters) were either absent or very few in number in the intestinal flora or feces. The SS mice, on the other hand, consistently had the typical lactose fermenters in their flora. These were tentatively identified as strains of *E. coli*.

Dubos and Schaedler infected the intestinal tract of the NCS mice by contaminating the drinking water with either a homogenate of the intestine of the SS mice or a dilute culture of *E. coli* obtained from the feces of the SS mice. Following the initial contact, the NCS mice became persistent carriers of *E. coli*. The same results could

also be obtained by placing the NCS mice in contact with the SS mice for several days.

The investigators noted that the *E. coli* content of the intestine of the contaminated NCS mice remained high for the first two weeks following innoculation and then fell to the lower level noted in the SS mice. This led them to conclude that the *E. coli* caused an infection in the intestine of the NCS mice rather than merely a multiplication of the microorganism in the intestinal contents.

Nutritional studies indicate that the NCS mice contaminated with either the intestinal contents or a culture of *E. coli* derived from the SS mice progressively acquire the growth characteristics of SS mice of the same sex and age. Following a single day of contamination with *E. coli*, NCS mice were placed on one of two diets: mouse pellets or whole grain corn. The animals so treated had growth rates similar to the SS mice, *i.e.*, the SS and NCS mice fed the pellets gained 7.8 and 7.1 g., respectively. The SS mice on the corn diet lost 3.4 g. and the contaminated NCS mice lost 3.6 g. over the three-week period. In comparison, the NCS mice not contaminated with *E. coli* had average weight gains on the pellet and corn diet of 8.2 and 1.0 g., respectively.

In another series of studies, contaminated and noncontaminated NCS mice were maintained on either a 15 per cent casein or a 15 per cent gluten diet and, in all cases, the growth rate of the noncontaminated NCS mice was greater (for the casein diet, 9.2 as compared with 7.0 g.; and for the 15 per cent gluten diet, 7.5 compared to 4.8 g.). Thus by infecting the NCS mice with the *E. coli*, which is a normal member of the intestinal flora of the SS mice, the growth characteristics of the NCS mice were changed to those of the SS group.

The possibility exists that, because of the small number of animals used in forming the NCS colony (12 mice), the two groups of mice may be genetically dissimilar. However, one cannot suggest that these differences have a genetic basis since it was shown that the intestinal flora of the two groups of animals were significantly different. The mechanism by which the different microflora influence growth cannot at present be explained.

IRRADIATED BEEF AND VITAMIN K

The hemorrhagic diathesis of rats fed irradiation-sterilized beef results from low vitamin K in irradiated beef and is prevented by oral supplementation with vitamin K_1.

Irradiation as a method for sterilizing foods has attracted much interest during recent years. For preparation and storage, this process has many advantages over conventional methods of food preservation. However, before commercial application, the wholesomeness and nutritive value of irradiation-sterilized foods must be proved by animal experimentation. An illustration of how animal experimentation can furnish such proof is afforded by studies concerning the nature of the hemorrhagic disease induced in rats fed irradiated beef.

Rats fed beef sterilized by irradiation develop a hemorrhagic tendency which can be prevented by oral administration of menadione (V. C. Metta, M. S. Mameesh and B. C. Johnson, *J. Nutrition* **69,** *18 (1959)*). It is possible that this diathesis is caused by hemorrhagic compounds or vitamin K antagonists produced in the beef during irradiation, since it is thought by some that, in the rat, intestinal microbial synthesis supplies adequate amounts of vitamin *K* (*Nutrition Reviews* **3,** *35, 48 (1945)*). However, the rat is uniformly susceptible to dietary vitamin K deficiency without the use of antagonists if coprophagy is prevented (*Ibid.* **18,** *306 (1960)*). Therefore, the results are also explicable on the basis of a dietary deficiency

of vitamin K caused by its lack in the irradiated beef.

The mechanism by which irradiated beef elicits a hemorrhagic response in rats has been recently investigated (Mameesh and Johnson, *J. Nutrition* **71,** *122 (1960)*). These authors used weanling, male albino rats of the Sprague-Dawley strain. They were individually housed in wire-bottomed cages and coprophagy was prevented by the use of tail cups.

In the first experiment, the effect on the hemorrhagic tendency of feeding different levels of irradiated beef was tested. Ground beef irradiated at 2.79 million rad was incorporated into an otherwise synthetic diet at levels of 42.0, 21.0 and 10.5 per cent of total dry matter. The amounts of protein and fat in each diet were kept approximately constant by adding soy protein and corn oil to those containing lesser amounts of irradiated beef. Vitamin supplementation was complete except for vitamin K. Groups of eight animals each were fed the diet for three weeks. Hemorrhagic deaths were confirmed by autopsy.

In the group fed the highest level of irradiated beef, two animals died with a hemorrhagic tendency, whereas at the two lower levels, six of the eight animals in each group died. In the groups fed the diets containing the two higher levels of irradiated beef, hemorrhagic deaths occurred only during the third week, whereas deaths occurred as early as the first week in the animals fed the diet with the lowest level of irradiated beef.

Thus the results of these experiments indicated that irradiated beef did not contain a hemorrhage-inducing compound, since the manifestations of hemorrhagic tendency increased in severity as the dietary level of irradiated beef was decreased. Furthermore, the results also indicated that the soy protein and corn oil used to equalize the protein and fat content of the diets were a poorer source of vitamin K than was irradiated beef.

In a second experiment, the authors determined the level of vitamin K necessary for the maintenance of normal blood coagulation in rats fed diets containing irradiated beef. A diet similar to that used in the first experiment and containing 35 per cent beef irradiated at 5.58 million rad was used. Vitamin K_1 (3-phytyl menadione) was given orally at levels of 0, 0.05, 0.10, 0.20, or 0.40 mg. per gram dry weight of diet. These supplements were incorporated into the diet at the time it was prepared (up to two weeks prior to use) or were given orally with a calibrated dropper.

Groups of five or ten animals were fed the diet for a period of four weeks, at which time they were sacrificed and the plasma prothrombin time determined. Supplementation of the diet with 0.1 mg. per gram or more of vitamin K_1 maintained the prothrombin time at normal levels, whereas lesser amounts failed to have this effect. The value for the minimum requirement of vitamin K_1 was the same as that reported for similar animals fed a purified diet (Mameesh and Johnson. *Proc. Soc. Exp. Biol. Med.* **101,** *467 (1959)*).

As the authors conclude, these results indicate that the hemorrhagic diasthesis produced in young male rats fed a semi-synthetic diet containing irradiated beef results from a lack of vitamin K and not from hemorrhagic compounds or vitamin K antagonists. Instead of producing the latter substances, the irradiation process apparently destroys or inactivates vitamin K contained in native beef. This report, moreover, prompts additional evidence that vitamin K is necessary in the diet of rats if coprophagy is prevented.

The necessity of thorough testing of foodstuffs produced by new or unusual processes is demonstrated by this study. It illustrates how possible inadequacies may be detected by animal experimentation and shows further that some of these may be corrected by supplementation with a single nutrient.

ABSORPTION OF AMINO ACIDS

It is demonstrated that L*-amino acids are actively transferred across the intestine from the mucosa to the serosa and that vitamin* B_6 *(pyridoxine) is necessary for this transfer.*

Several groups of investigators have attempted to elucidate the mechanisms of amino acid transport through the intestine. Adding to the original findings of R. Hoeber and J. Hoeber (*J. Cell. Comp. Physiol.* **10,** *401 (1937)*) and of F. A. Schoefield and H. B. Lewis (*J. Biol. Chem.* **168,** *439 (1947)*), G. Wiseman (*J. Physiol.* **127,** *414 (1955)*; **133,** *626 (1956)*) employed the everted sac method to demonstrate that L-amino acids are able to pass through the membrane of the intestine of the rat not only by free diffusion but also by some active mechanism against a concentration gradient. D-Amino acids were shown to be transported only by free diffusion.

W. T. Agar *et al.* (*Biochim. Biophys. Acta* **22,** *21 (1956)*) observed that 2,4-dinitrophenol diminished the concentration of L-amino acids in small pieces of segmented intestine, but had no effect with D-amino acids. L. Fridhandler and J. H. Quastel (*Arch. Biochem. Biophys.* **56,** *424 (1955)*) observed an inhibitory effect of deoxypyridoxine on L-amino acid transfer, and F. A. Jacob and R. S. L. Hillman (*J. Biol. Chem.* **232,** *445 (1958)*) showed by means of an *in situ* technique that the rates of absorption of both L- and D-methionine were reduced by the addition of deoxypyridoxine.

During the past year a report has been issued from the Institute for Protein Research, Osaka University, Japan (H. Akedo, T. Sugawa, S. Yoshikawa and M. Suda, *J. Biochem.* **47,** *124 (1960)*) in which both *in vivo* and *in vitro* experiments on absorption of amino acids by rat intestine are described. These studies provide evidence for a role of vitamin B_6 in the transfer of L-amino acids during active absorption.

Young male albino rats weighing 120 to 150 g. were fasted for 30 hours before the experiment. The section of the gut from the jejunum to the ileocecal region was divided into two equal lengths and placed in an *in vivo* circulation apparatus so that Krebs-Ringer bicarbonate buffer containing 0.2 per cent glucose and the amino acids under study could be run through the intestinal canal while the blood supply was still intact. In the case of *in vitro* experiments the apparatus reported by R. K. Crane and T. H. Wilson (*J. Applied Physiol.* **12,** *145 (1958)*) was used.

With this latter method and S^{35} DL-methionine, the rate of amino acid transportation from the mucosa to the serosa and vice versa were tested. When the everted sac was used, the transfer of amino acids from the mucosal to the serosal solutions could be measured. At the start of the experiment, the concentrations of both L- and D-methionine on each side of the intestine were equalized by the addition of non-radioactive L- *or* D-methionine, respectively. If the transfer mechanism for each isomer was the same, the amounts of each isotope inside the sac would be almost equal, whether the experiments were carried out in the presence of the L or the D carrier. However, the rate of appearance of isotope inside the sac (mucosa to serosa) was much faster when D-methionine was used as a carrier.

These results were interpreted as indicating that the L-isomer is actively transported while transfer of the D-isomer is by simple diffusion. Transfer of DL-methionine from the serosa to the mucosa is much slower than in the reverse direction. This is interpreted to mean that from serosa to mucosa the transfer of both isotopes is probably by free diffusion. Dinitrophenol (10^4 M) suppressed the rate of active absorption of L-amino acids.

In the *in vivo* experiments, 10 mg. of L-penicillamine per 100 g. body weight were

injected twice during a ten-hour interval, inducing a deficiency of vitamin B_6. During the circulation of the amino acid solution through the lumen, 500 μg. of pyridoxine were administered through the caudal vein and disappearance of amino acids from the circulation fluid was measured as a function of time.

The rate of disappearance of only L-amino acids (histidine, lysine and methionine) was markedly accelerated by injection of pyridoxine. From these results the investigators concluded that in vitamin B_6 deficiency, the rate of absorption of L-isomers is decreased relative to that of the D-isomer and is restored by vitamin B_6 to the normal rate. The ineffectiveness of pyridoxine toward D-isomers is associated with the fact that D-isomers are absorbed by simple diffusion.

No effect of pyridoxine on the absorption of glucose could be detected, and vitamins B_1 and B_2 were unable to substitute for vitamin B_6 in the absorption of L-amino acids.

The authors were able to show that rats pretreated with L-penicillamine were actually in a state of B_6 deficiency. Knowing that xanthurenic acid is excreted in the urine of vitamin B_6-deficient rats following the administration of tryptophan, they challenged L-penicillamine-treated rats following injections with L-tryptophan and noted a subsequent excretion of xanthurenic acid in these animals. Moreover, it has been shown that 4-pyridoxic acid represents the major end product of vitamin B_6 excreted in the urine. After L-penicillamine treatment, the 4-pyridoxic acid level of the urine of rats fell from 33 μg. per day per rat to less than 10 μg. during the same period.

FAT DEFICIENCY AND PITUITARY-GONAD RELATIONSHIPS

Dietary fat deficiency in the rat results in degeneration of the seminiferous tubules. The deficiency probably depresses the production of testicular androgen and the primary site of action may be at the pituitary or testicular level.

Impaired reproductive ability was one of the first defects noted in the fat-deficient rat (G. O. Burr and M. M. Burr, *J. Biol. Chem.* **86,** *587 (1930)*). Arrest of spermatogenesis with degeneration of the seminiferous tubules has been noted in the testicles of fat-deficient rats (H. M. Evans, S. Lepkovsky and E. A. Murphy, *Ibid.* **106,** *445 (1934)*). The effects of fat deficiency on gonadal structure and function are of great interest.

Some factors, such as inanition, probably act nonspecifically by a general interference with growth, whereas others apparently have a more direct action on the pituitary-gonadal axis. The pituitary may fail to produce or secrete gonadotrophins with a resultant secondary degeneration of the testes and accessory sex organs. A primary effect at the testicular level may be a result either of failure of interstitial cells to secrete androgens or of a specific tubular degeneration. In these latter cases, so-called castration changes occur secondarily in the pituitary.

Failure of production of pituitary gonadotrophin has been described in fat-deficient rats (S. M. Greenberg and B. H. Ershoff, *Proc. Soc. Exp. Biol. Med.* **78,** *552 (1951)*). These authors reported reversal of the atrophy of secondary sexual structures after administration of chorionic gonadotrophin. Histologic evidence of gonadotrophin insufficiency has also been reported in fat-deficient rats (T. C. Panos and J. C. Finerty, *J. Nutrition* **49,** *397 (1953)*, **54,** *315 (1954)*).

In a recently reported study, the effects of dietary fat deficiency on pituitary-gonadal relationships are described further, and a careful attempt is made to determine the primary locus of action (Panos, G. F. Klein and Finerty, *J. Nutrition* **68,** *509 (1959)*).

In this study, 250 male weanling, Holtzman albino rats were employed. The animals

were separated into the following five groups according to diet: (1) those fed ad libitum the basal diet free of fat, but adequate in other respects; (2) those pair-fed the basal diet supplemented with cottonseed oil to provide 30 per cent of the calories at the expense of carbohydrate so that the daily intake of calories was equivalent to that of group one; (3) those fed ad libitum the basal diet supplemented with cottonseed oil; (4) those fed the basal diet supplemented with cottonseed oil in an amount such that body weight was equal to that of animals fed the fat-free diet (group one); and (5) a group fed laboratory chow ad libitum.

By using this combination of dietary regimens, differences caused by variations in caloric intake were ruled out by group two and differences due to the effects of inanition by group three. Group four served as a control for any abnormality in either absorption or utilization of nutrients induced by the deficiency, while group five served as a "normal" dietary control.

Animals in groups one and three were sampled after nine, 12, and 15 weeks on their respective dietary regimens, and the pituitaries, testes and accessory sex organs were examined grossly and histologically. Significant testicular degeneration was noted in group one after 15 weeks on the diet.

In order to determine the effect of replacement hormonal therapy on testicular degeneration, three subgroups of eight animals each from group one were treated with the following, beginning at 18 weeks: (a) testosterone, 100 mg.; (b) chorionic gonadotrophin, 2 units; or (c) pituitary tissue in saline solution, 0.6 mg. (most of the activity of the latter is said to be follicle stimulating hormone). Animals were injected daily for two weeks, at the end of which time they were sacrificed along with noninjected controls from groups two, three, four, and five. At 20 weeks, similar injections were begun in all the remaining animals except those in group five.

For histologic examination, testes, prostate, and seminal vesicles were fixed in Bouin's solution and stained with hematoxylin and eosin. Pituitary glands were fixed in formol-saline and stained by a periodic acid-Schiff methyl blue (PAS-MB) technique which distinguishes two types of basophiles, a red and a purple, which are supposely gonadotrophic cells correlated with production of luteinizing hormone and follicle-stimulating hormone, respectively (J. E. Hildebrand, E. G. Rennels, and Finerty, *Zeitschr. f. Zell forschung.* **46,** *400 (1957)*).

The animals fed the fat-free diet gained less body weight, and both testicular and prostate weights were less than the controls. Although the ratio of testicular to body weight was less than in the normals, this ratio was about the same for the prostates. The seminal vesicles in the fat-free group were about normal weight. In general, these changes were detectable by the ninth week and increased with the progress of the experiment.

At 20 weeks, the chow-fed animals had greater body, testes, prostate and seminal vesicle weights than did any of the other controls. Animals fed the fat-supplemented basal diet, isocalorically paired with the fat-free group or ad libitum, had greater body, testes, and accessory weights than did animals fed the fat-free diet. The testes obtained from those animals fed the basal diet supplemented with fat at a level such as to maintain body weight equivalent to that of animals fed the fat-free diet were significantly smaller than in those fed the supplemented diet isocalorically with the fat-free group or ad libitum.

When injected into the fat-free group from the eighteenth to the twentieth week, chorionic gonatrophin and pituitary tissue in saline solution stimulated the accessory sex organs but had no effect on testicular weight. Testosterone decreased the testicular weight (as it did in controls) and had no effect on accessory sex organs. None of the hormones affected body weight at this time.

Hormonal injections in rats fed the deficient diet for 20 to 23 weeks produced more striking changes. Testosterone caused a significant increase in body weight, testicular weight, and weights of accessory sex organs. Only the increase in testicular weight was greater than the rate of increase of body weight. Chorionic gonadotrophin and pituitary tissue in saline solution stimulated the accessory sex organs without significantly affecting either body weight or testicular weight.

Histologic examination of the testes of the fat-free animals showed a progressive degeneration of seminiferous tubules similar to that described in vitamin E-deficient rats (K. E. Mason, *Am. J. Anatomy* **52,** *753 (1933)*). Minimal changes were detectable by the ninth week. Early changes consisted of cytoplasmic vacuolization and decrease in tubular size. Subsequently, there was loss of spermatozoa from tubules with formation of abnormal spermatids which fused to form multinucleated giant cells.

By the twentieth week all testicles from the fat-free group showed degenerative changes to some degree and about one fourth to an extreme degree (giant cells). None of the three hormonal preparations at this time had any effect on tubular structure. At the twenty-third week, advanced changes were more common (in about one half). Testosterone injection at this time caused the appearance of many spermatozoa and secondary spermatocytes. Chorionic gonadotrophin apparently had a slight effect in that fewer tubules showed extreme degenerative changes than those in the deficient controls, but pituitary preparation had no effect.

Of the controls, only the group paired by body weight against the fat-free group showed any changes caused by the dietary regimen or by hormonal replacement. In this group, at 20 to 23 weeks there was minimal tubular degeneration but no giant cell formation or extreme atrophy. Injection of testosterone in this group had no effect, whereas pituitary preparation appeared to cause some improvement and chorionic gonadotrophin potentiated the degenerative changes.

No apparent differences in quantity of interstitial cells were noted between control and fat-deficient groups. In the latter, cells with "wheel" nuclei characteristic of gonadotrophin deficiency were noted. Chorionic gonadotrophin appeared to cause some stimulation in number and activity of interstitial cells.

By nine weeks, the total number of pituitary basophiles in the fat-free group was decreased with a normal predominance of cells stained purple by the PAS-MB technique. A few "castration" cells were present at this time. Between 12 and 15 weeks there was a progressive increase in the number of "castration" cells and in the proportion of red-stained to purple-stained cells. By the twenty-third week, only occasional purple-stained cells were found whereas red-stained cells were numerous. Pituitaries from animals in the control groups were cytologically normal. At 20 weeks testosterone injections administered to rats in the fat-free group caused a reversal to normal of the cytologic abnormality described. Chorionic gonadotrophin reduced the size and apparent activity of gonadotrophic cells while pituitary tissue in saline exerted no effect.

In essence, absence of dietary fat appeared to have been associated with a deficiency of androgen. However, as the authors point out, whether this was brought about by an inability of the interstitial cells to secrete androgen in the face of normal or elevated gonadotrophin or by failure of secretion of pituitary gonadotrophin could not be distinguished by this elaborate and carefully conducted experiment in intact animals. Testosterone caused the most significant restorative effect and this result might seem to indicate a primary hypogonadism. However, chorionic gonadotrophin caused similar but less extensive changes, indicating that the interstitial cells were capable of at least partial function when greatly stimulated.

This carefully controlled study illustrates

the sensitivity of the pituitary-gonad axis to a specific nutritional deficiency. As an extension of this report, gonadotrophin assay of pituitaries obtained from animals maintained on the described regimens may help to elucidate the relative roles of the pituitary and gonad in the reproductive ability of fat-deprived rats.

VITAMIN E DEFICIENCY IN THE GUINEA PIG

Torula yeast, which enhances certain symptoms associated with a vitamin E deficiency in rats, chicks and mice, appears to delay or prevent muscular dystrophy in the guinea pig, even though the yeast contains no vitamin E.

The knowledge that fairly high levels of Torula yeast as a source of protein in a diet help produce a vitamin E deficiency in rats, mice and chicks which can be overcome by vitamin E (*Nutrition Reviews* **16,** *149, 174 (1958)*) led to the question of whether this yeast might not enhance a vitamin E deficiency in other species. With this in mind, J. C. Seidel and A. E. Harper (*J. Nutrition* **70,** *147 (1960)*) fed week-old guinea pigs weighing 400 g. various diets from which vitamin E had been omitted. On the purified 30 per cent casein diet, the animals developed symptoms of muscular weakness within a period of eight to 11 weeks. The rate of body weight gain for the animals on the deficient diet and on that supplemented with adequate amounts of vitamin E were the same for the first eight weeks of the experiment. From then on, the vitamin E-supplemented animals continued to gain weight, whereas the animals on the deficient diet started to lose.

When a similar diet was used (containing 20 per cent casein and 20 per cent Torula yeast as sources of protein) the vitamin E-supplemented animals and those on the deficient diet showed the same rate of body weight gain over a 15-week period. Their growth was similar to that of the vitamin E-supplemented rats on 30 per cent casein. The animals on the deficient diet containing no Torula yeast showed creatinuria and muscular weakness beginning at the eighth week. However, those animals receiving the vitamin E-deficient diet containing Torula yeast showed neither creatinuria nor muscular dystrophy through the sixteenth week, the end of the experiment. In spite of the evidence suggesting that Torula yeast, under these circumstances, had a vitamin E-sparing action, the addition of Torula yeast to the diets of two vitamin E-deficient guinea pigs had no effect in alleviating the deficiency symptoms.

The rabbit, which also shows severe muscular dystrophy when fed a vitamin E-deficient diet, responds only to vitamin E. Although Factor 3-active selenium compounds are much more active on a weight basis than vitamin E in preventing dietary liver necrosis and exudative diathesis in chicks (*Nutrition Reviews* **18,** *193 (1960)*), this element has no effect on muscular dystrophy in the rabbit fed a vitamin E-deficient diet. E. L. Hove, G. S. Fry and K. Schwarz (*Proc. Soc. Exp. Biol. Med.* **98,** *27 (1958)*) produced acute muscular dystrophy within three weeks in four-week-old male rabbits that were fed a soybean meal diet containing 6 per cent vitamin E-free lard and 2 per cent cod liver oil.

Addition of selenium, as sodium selenite or selenocystine, to the above diet had no effect on the muscular dystrophy. Activation analysis (production of specific radioisotopes of selenium by neutron bombardment and subsequent carrier analysis) of the soybean meal indicated the presence of 0.3 μg. of selenium per gram. The 40 per cent soybean meal in their ration, therefore, provided 12 μg. of selenium per 100 g. of ration. This is a very high level

compared with the rat's requirement for the element.

Preliminary experiments by Seidel and Harper showed no effect by sodium selenite on muscular dystrophy in the guinea pig, in accordance with the fact that casein is usually a good source of Factor 3-selenium. Since Torula yeast is a good source of coenzyme Q, the authors injected coenzyme Q, isolated from Torula yeast, but this did not alleviate muscular dystrophy in vitamin E-deficient guinea pigs.

THIAMINE AND LIVER LIPIDS

Thiamine deficiency in rats resulted in low levels of liver neutral glycerides, but cholesterol, plasmalogens and other phospholipids tended to be maintained at high levels.

A number of studies have been concerned with the relationship of thiamine deficiency to lipid metabolism (E. W. McHenry, *J. Physiol.* **85,** *343 (1935)*; **89,** *287 (1937)*; McHenry and G. Gavin, *J. Biol. Chem.* **125,** *653 (1938)*; and Gavin and McHenry, *Ibid.* **132,** *41 (1940)*). These studies have indicated that as thiamine stores in the rat are exhausted, a double deficiency of choline and thiamine occurs and liver lipid decreases markedly. With a choline deficiency alone, a fatty liver condition obtains; thus production of a fatty liver in choline deficiency appears to be dependent on the presence of thiamine.

When rats are starved and then refed (F. Rubino *et al.*, *Bull. Soc. ital. Biol. sper.* **32,** *36 (1956)*) there is a greater deposition of total body fat when thiamine is included in the diet. R. W. Engel and P. H. Phillips (*J. Nutrition* **18,** *329 (1939)*) have shown that treatment with thiamine in thiamine-deficient chicks produces fat globules in the parenchyma of the liver. It thus appears that thiamine may have a more directly significant role in lipid metabolism than simply to promote fat deposition by increasing appetite or assisting in the production of lipid from carbohydrate.

J. N. Williams and C. E. Anderson (*J. Nutrition* **69,** *229 (1959)*) have suggested that thiamine may be involved in fat mobilization and storage by an unknown mechanism. A great deal of their interest has centered about the possible role that thiamine might play in maintaining liver plasmalogens. (The plasmalogens comprise a group of phospholipids which give rise to long chain aldehydes on acid hydrolysis, the aldehyde group probably being attached to the glycerol moiety of the molecule at the alpha-carbon atom by a vinyl-ether linkage.) Although the metabolic source of the long-chain aldehydogenic component of the molecule is unknown, it seemed possible to relate it to the action of thiamine in carbohydrate metabolism by virtue of its function in the decarboxylation of pyruvate.

D. E. Green *et al.*, (*J. Biol. Chem.* **145,** *69 (1942)*) showed that a thiamine-containing enzyme is capable of decarboxylating not only pyruvate but also alpha-ketoglutarate acid and alpha-ketobutyrate. Williams, and Anderson (*loc. cit.*) pose, by analogy, a possible mechanism by which the aldehyde-yielding portion of the plasmalogen molecule could arise by similar decarboxylation of a long-chain keto acid. In a series of experiments, they followed the plasmalogen concentration of liver during the development of a thiamine deficiency to determine whether the production of this component is influenced by thiamine.

One group of 26 rats was fed a purified ration consisting of 20 per cent casein, 0.3 per cent DL-methionine, 5 per cent corn oil, 4 per cent salts, 2 per cent vitamin mix and sucrose to make 100 per cent. A second group of 74 rats was fed the same ration except that thiamine was omitted. At four- to five-day intervals after placing the animals of group two on the thiamine-

deficient ration, six rats were sacrificed and liver phospholipid, total lipid and plasmalogens were estimated. Total cholesterol of the lipid extracts was determined beginning with the eighth group of thiamine-deficient rats. The animals receiving the complete ration were sacrificed in groups of six after zero, 18, 44 and 55 days. On day 49 the remaining thiamine-deficient rats were injected intraperitoneally with 1 mg. of thiamine. After two and four hours, two groups of three rats each were sacrificed. After 48 hours, the last four animals were sacrificed.

The total liver lipids in the thiamine-deficient rats began to decrease immediately after the start of the diet. By the 31st day the livers had been almost completely depleted of neutral lipid. Following thiamine treatment (forty-ninth day) there was a sharp rise in total lipid to a high normal level after 48 hours. Over the 48-hour period, liver weights increased 2.5-fold and changed from a deep red to a light tan color. The iodine numbers for this group averaged 84 as compared with 75 for the normal group and 121 for the untreated animals of the thiamine-deficient group. When calculated for 100 g. of body weight, the liver phospholipids in the thiamine-deficient animals were lower than in the controls. Thiamine injection caused an increase in liver phospholipids with respect to total body weight.

The plasmalogens followed much the same pattern as the other phospholipids with the exception that they were held most tenaciously, remaining constant with respect to body weight. Per gram of liver, a significant increase occurred as thiamine deficiency developed. Cholesterol values followed more nearly the pattern of the phospholipids than of the total lipids.

Thus the original notion of the authors that the long-chain aldehydrogenic component of the plasmalogens may arise from decarboxylation of the long-chain keto acids is not substantiated by the present experiments. There was no tendency for a decreased synthesis of the plasmalogens. The sudden decrease in plasmalogen when thiamine was injected was explained by the authors on the same basis as that of the other phospholipids, namely, a lack of stimulation of plasmalogen synthesis by thiamine coupled with increased deposition of neutral lipid and other liver components.

INHIBITION OF CORTISOL-INDUCED GLUCONEOGENESIS BY PYRIDOXINE DEFICIENCY

Cortisol increases hepatic glutamic-pyruvic transaminase and causes hepatic glycogen deposition. A deficiency of pyridoxine blocks this effect but does not prevent atrophy of the spleen and adrenals.

All of the anti-inflammatory corticosteroid hormones have a major effect upon carbohydrate metabolism; hence the name "Glucocorticosteroid." These hormones induce hyperglycemia and glycogen deposition simultaneously, a feat which is accomplished by conversion of other substances (principally amino acids) into glucose. The enzyme responsible for this conversion, glutamic-pyruvic transaminase, is found chiefly in the liver. Cortisol has been shown to enhance the activity of this enzyme (F. Gavosto, A. Pileri, and A. Brusca, *Biochim. Biophys. Acta* **24,** *250 (1957)*), yet other adrenal steroids lack this action (F. Rosen, N. R. Roberts, L. E. Budnick, and C. A. Nichol, *Endocrinology* **65,** *256 (1959)*).

A. B. Eisenstein (*Endocrinology* **67,** *97 (1960)*) reasoned that if the carbohydrate effect of cortisol depends upon increased activity of hepatic glutamic-pyruvic transaminase, a deficiency of pyridoxine might block this action, since pyridoxal phosphate is the cofactor of this enzyme. Accordingly, he compared the effect of cortisol in normal

and in pyridoxine-deficient rats with other groups of normal and pyridoxine-deficient animals given injections of a placebo. Measurements were made of total body weight, splenic weight, adrenal weight and hepatic weight. Determinations were made of hepatic glycogen and of the amount of glutamic-pyruvic transaminase formed per hour by a sample of fresh liver.

The results indicated that either a deficiency of pyridoxine or the administration of cortisol retarded the rate of weight gain despite pair-feeding. Splenic weight and adrenal weight decreased in both deficient and in normal groups when cortisol was given (both effects would be expected from this hormone). The weight of the liver doubled in normal animals given cortisol, and this increase was accompanied by a profound increase in hepatic glycogen and a similar exaggeration of glutamic-puruvic transaminase activity. However, in pyridoxine-deficient animals hepatic weight remained almost unchanged when cortisol was given. There was much less glycogen deposited in the liver (four times control values compared with 18 times) and the glutamic-pyruvic transaminase activity increased modestly.

This study demonstrates that one of the important effects of pyridoxine is related to the gluconeogenic effect of cortisol through augmentation of the activity of glutamic-pyruvic transaminase. It further demonstrates that one effect of cortisol can be blocked, in this instance by removing the source of pyridoxal phosphate, while the other effects remain as evidenced by the fact that lympholysis resulted in a decrease in splenic weight and that inhibition of the pituitary adrenocorticotrophic hormone resulted in a decrease in adrenal weight.

This study was simple, direct and conclusive. It demonstrated the interrelation between a vitamin, a hormone and an enzyme in a most convincing manner.

RESTRICTION OF FOOD OR WATER AND WORK OUTPUT

Contrary to belief, the most important factor limiting work output is not the restriction of food, but the restriction of water.

Many believe that the restriction of food is the primary factor limiting an individual's work performance. It is frequently assumed that a reduction in food intake, even for a short time, results in a reduced ability to perform physical labor. This concept is also inherent in the definition of starvation, which usually implies a deprivation of food in the presence of an unlimited supply of water.

The influence of even a short period without food has been studied by W. W. Tuttle and his collaborators. They found that when either school boys or college girls went without breakfast there was a reduction in physical efficiency toward the end of the morning (*Nutrition Reviews* **15,** *196 (1957)*). The deterioration was assessed on the basis of measurements of neuromuscular tremor, choice reaction time, and maximum work output. The latter was determined by the energy expended during one minute of exhausting work on a bicycle ergometer. Most of the work was done with the college women who on an average showed a reduction in response when breakfast was omitted. There was, however, considerable individual variation in the group, with some of the women showing no change in performance when breakfast was omitted.

Of much greater significance from the standpoint of work output is the effect of water restriction. This area has been studied by D. R. Young and co-workers. They recently reported on the relation between the time lapsed since the last meal and the

efficiency of work output (*J. Applied Physiol.* **14,** *1013 (1959)*). They extended these studies to evaluate the relative effect of water and of food on work efficiency.

Six highly trained adult beagle dogs weighing 8.4 to 12.1 kg. were used in all experiments. When the dogs were exercised on a motor driven treadmill for one and one-half, four, six, or 24 hours following the ingestion of 250 g. of a commercial chow, there was no change in mean energy expenditure, respiratory quotient, or mean pulse rate if the run was made as late as six hours after the meal. There was a reduction in respiratory quotient from 0.93 to 0.79 when the run was made 24 hours after the last meal. Apart from the latter, the dogs responded at 24 hours as they did at one and one-half hours after the meal to a 40-minute run on the treadmill at a speed of 3.6 miles per hour and at grades varying from 9.4 to 12.5 degrees. The grade was adjusted so as to produce a constant work intensity of 203 kg. meters per minute for each dog. In these studies, water was available to the dogs at all times except during the run on the treadmill.

It was observed that the body weights of the dogs showed characteristic fluctuations throughout the day. By approximately four hours after eating, the maximum weight was attained. This was presumably associated with the ingestion of water following the food that had been eaten. During the next two hours there was a rapid reduction in body weight amounting to 5 per cent. For the remainder of the 24 hours (during which no additional food was given) the weight loss was more gradual and during this interval it again amounted to 5 per cent, producing a 10 per cent overall change in weight. Young and co-workers (*loc. cit.*) calculated that the variations in body weight represented changes in body water varying, each day, from 65 per cent at the time of maximum weight to 57 per cent at the time of minimum weight.

To evaluate the relative effect of water and carbohydrate supplementation on work performance, six dogs were run to exhaustion on a treadmill at a rate of 3.6 miles per hour and a grade of 10 per cent. These runs were made 17 hours after the last meal. Exhaustion was assumed to have set in when the animal failed to maintain his speed after six successive shocks from an electric stimulator mounted at the rear of each treadmill.

When the dogs were run to exhaustion without receiving any supplement, they were able to continue until they expended 1191 calories. The post-exercise glucose level in these dogs was 40 mg. per 100 ml. of whole blood, whereas the pre-exercise value had been 79.

To evaluate the effect of a carbohydrate supplement on work output, these dogs were put through the same test except that 30 minutes prior to the start of the exhaustive run they received a carbohydrate pellet equal to 0.5 per cent of the dog's body weight. The dogs ran until they expended 1299 calories and showed a reduction in blood glucose to 59 mg. per 100 ml. This reduction and a respiratory quotient of 0.85 were the only differences attributable to the carbohydrate supplement. The respiratory quotient in the control dogs receiving no food during the 17 hours preceding the run was 0.77. The significance of the higher respiratory quotient following the carbohydrate supplement is not apparent since no tests for ketosis were performed.

In the third part of the experiment, the dogs were permitted to drink water during the run. Water troughs were attached to each side of the treadmill so that the dogs could drink while running. Each dog consumed approximately 1.5 liters of water at this time, and as a result increased his endurance so that he could run until he expended 2141 calories. The 80 per cent increase in working ability associated with water intake during the run was highly significant ($P < .01$). The ingestion of water was associated with only a 14 mg. per 100 ml. reduction in blood glucose during the work and a maximal rectal temperature of 104.2° F. compared to 105.4 and 105.7 in the

control and carbohydrate-supplemented groups respectively.

The dogs receiving the water showed a loss of body weight equal to 6.9 per cent ± 0.77 of the pre-exercise value. The losses in weight for each of the other two groups were 11.2 ± 1.7 and 11.1 ± 1.1 per cent.

Water supplementation produced a marked improvement in endurance capacity. The drinking of water during the run was of much greater importance than the ingestion of carbohydrate just prior to the work task. The investigators suggest that water supplementation maintains a relatively normal state of hydration and has a beneficial effect on carbohydrate metabolism. The latter was shown by the relatively slight change in blood glucose levels produced by the work when the dogs drank during the run.

The lapse of four to six hours following the ingestion of a substantial meal produces no measurable deterioration in work performance of dogs as determined by a run on a treadmill. A period as long as 24 hours after the last meal has only a minimal effect on work as shown by a reduction in the respiratory quotient during work.

The importance of an adequate intake of water as a means of preventing fatigue has been stressed by O. Mickelsen (*U. S. Department of Agriculture Yearbook, p. 168 (1959)*). He points out that fatigue is the first symptom of even a mild dehydration resulting from the loss of sweat during work. The fatigue may become so severe, if the water loss is great, that the individual is unable to work.

The significance of an adequate intake of water was emphasized by J. Hunt in *A Conquest of Everest, E. P. Dutton and Co. Inc., New York (1954)*. When Sir Edmund Hillary was preparing for the ascent of Mount Everest, he reviewed the records of the Swiss expedition which attempted the ascent a year earlier. He concluded that the climbers suffered from extreme fatigue because their water consumption was less than one pint a day. This was totally inadequate to maintain hydration under the conditions of high altitude and the hard work associated with mountain climbing. The British expedition made provision for a water consumption of five to seven pints per person per day. Hillary attributed the success of his expedition largely to the maintenance of normal hydration during the last few days of the ascent of Mount Everest.

These studies of Young and co-workers again emphasize the tremendous nutritional and physiological importance of water.

DIETARY FATS AND ATHEROSCLEROSIS IN CHICKENS

The nature of the dietary fat is important in the deposition of fat in the aorta of cholesterol-fed chickens. Its influence on deposition may be independent of any effect on blood cholesterol.

It has been demonstrated by a number of investigators that the feeding of various plant and marine fats to men will lower the concentration of cholesterol in their serum or plasma (L. W. Kinsell *et al., J. Clin. Nutrition* **1,** *224 (1953)*; E. H. Ahrens, Jr. *et al., Proc. Soc. Exp. Biol. Med.* **86,** *872 (1954)*; B. Bronte-Stewart *et al., Lancet* **I,** *521 (1956)*; and H. Dam *et al., Acta Physiol. Scandinav.* **45,** *31 (1959)*). It has been suggested that this effect may be related to the source of fat (J. M. R. Beveridge *et al., J. Nutrition* **56,** *311 (1955)*), its iodine value (Ahrens *et al., Lancet* **1,** *543 (1957)*), the specific types of unsaturation in the constituent fatty acids, the chain lengths of the fatty acids and the presence of other substances such as betasitosterol.

Other investigators have compared the influence of various fats on cholesterol concentrations and on vascular lipid deposits in chickens, rabbits, rats and monkeys with

results that have often differed from one another and from results in man (*Nutrition Reviews* **18,** *21* (*1960*); **17,** *10* (*1959*); **16,** *68*, *83*, *131* (1958)).

A study has been completed by D. M. Tennent and co-workers (*J. Nutrition* **69,** *283* (*1959*)) in which a comparison of the influence of several vegetable fats on plasma cholesterol concentrations and degree of aortic atheromatosis in cholesterol-fed and in diethylstilbestrol-implanted cockerels has been made. (Estrogen-treated birds display an increase in the low density serum lipoproteins that is primarily endogenous in origin. Such increases in light lipoprotein following estrogen treatment do not occur in man. In fact the tendency is toward a decrease in low density lipoproteins accompanied by an increase in high density lipoproteins.)

The fats studied were: cacao butter, olive oil, cottonseed oil, safflower oil, linseed oil and tung oil. These ranged in iodine value from 35 to 180.

In each experiment, 60 eight-week-old White Leghorn cockerels were divided into groups of ten and fed experimental rations containing corn meal, soybean meal, fish meal, alfalfa meal, adequate vitamin and mineral supplements and a given vegetable fat for eight weeks. The fats were fed as 5 per cent of the diet replacing an equal weight of corn meal in the basal ration. In the cholesterol-fed groups, 2 per cent U.S.P. cholesterol was substituted for an equal weight of corn meal. The diets of diethylstilbestrol-injected cockerels (15 mg. every ten days) contained no added cholesterol.

The nature of the fat in the diet of cholesterol-fed cockerels influenced the concentrations of lipids in the plasma. While no values were reported for total plasma lipid, lipid phosphorus data indicated that cottonseed oil, in spite of its 50 per cent linoleic acid content, uniformly produced high plasma lipid concentrations; cacao butter and olive oil also resulted in high concentrations, while linseed and safflower oils produced low lipid phosphorus values.

Plasma cholesterol levels were inversely proportional to the total percentage of polyenoic acids in the fat fed (or directly proportional to the saturated plus monoenoic acid fed). Plasma lipid phosphorus concentrations were inversely proportional to the total amount of unsaturation, but aortic lesion scores were not closely related to the composition of the fat fed. Cottonseed oil consistently produced a high lesion score but results with the other five oils did not differ sufficiently to indicate a trend. Among birds fed any given fat, those with the greatest plasma lipid concentrations also tended to be those with the greatest lesion score. The data also suggest that high plasma cholesterol concentrations in birds fed some fats such as olive oil or cacao butter may be less injurious than the same concentrations in birds fed other fats such as safflower or corn oils.

The authors consider that the atherogenic property of cottonseed oil is related to its content of one or more specific substances with this action that is not present to great degree in the other fats studied. In this connection, D. F. Opdyke and W. H. Ott (*Proc. Soc. Exp. Biol. Med.* **85,** *414* (*1954*)) have observed that different preparations of cottonseed oil differ with respect to atherogenic potency.

The cockerels implanted with diethylstilbestrol exhibited increased plasma cholesterol concentrations and greatly increased plasma lipid phosphorus concentrations but aortic lesions developed more slowly than in cholesterol-fed birds. The nature of the fat in the diet had no discernible influence on lesion scores or plasma lipid concentrations in implanted birds. The authors therefore conclude that the influence of the dietary fat on the lipid pattern depends on the mechanism by which the plasma lipids have been elevated.

Although these conclusions do not appear to agree with those reached from experi-

ments with mammalian or human subjects, it should be realized that the chicken appears to differ in other aspects of metabolism in general and lipid metabolism in particular. The results do agree quite closely with those of D. M. Hegsted *et al.* (*J. Nutrition* **70,** *119 (1960)*) who also performed this experiment with chicks.

IRON ENZYMES IN IRON DEFICIENCY

Iron deficiency resulted in a decrease in activity of succinic dehydrogenase in kidneys and hearts of rats but no change in the activity of this enzyme in the livers.

It has previously been demonstrated that several iron-containing enzymes are affected in iron deficiency. Under such circumstances, cytochrome C, catalase and cytochrome oxidase are depleted from some tissues but not from others (E. Beutler, *Am. J. Med. Sci.* **234,** *517 (1957)*; Beutler and R. K. Blaisdell, *J. Lab. Clin. Med.* **52,** *694 (1958)*; Beutler and Blaisdell, *J. Clin. Invest.* **37,** *833 (1958)*). It has recently been suggested that iron is a component of the enzyme succinic dehydrogenase, but, unlike the enzymes previously studied in iron deficiency, the iron in succinic dehydrogenase is apparently not present in the form of a heme group.

Beutler and Blaisdell (*Blood* **15,** *30 (1960)*) have undertaken the study of this enzyme under conditions of iron deficiency in the hope that some of the symptoms that occur in this deficiency state may be more satisfactorily explained.

In an initial experiment, four weanling rats were placed on an iron-poor diet for four weeks. At seven weeks of age they were sacrificed and body weight, hemoglobin, red cell count, hematocrit, plasma iron, liver weight and liver succinic dehydrogenase activity determined. Four other rats were given 12.5 mg. of iron dextran at three weeks and again at five weeks of age. These, too, were sacrificed at seven weeks of age and the same determinations were carried out.

The results indicated that a moderately severe degree of iron-deficiency anemia existed in the animals that had not received iron. Although the weights of the iron-deficient animals were also subnormal, the average liver succinic dehydrogenase activity was almost identical in the two groups. Even in an additional group of animals that had been iron deficient for 25 to 29 weeks, there was no decrease in liver succinic dehydrogenase activity.

In a study to determine whether heart and kidney succinic dehydrogenase activity was likewise unaffected by iron-deficiency, eight 14-week-old rats which had been on iron-poor diets for 11 weeks were sacrificed. In these animals, succinic dehydrogenase activity was markedly reduced in both hearts and kidneys. Eight additional rats showed the same reaction to iron deficiency, namely, a reduction of succinic dehydrogenase activity in both the heart and kidney but no change in the activity of this enzyme in the liver. The weights of the hearts in iron-deficient animals were increased to the extent that the total succinic dehydrogenase activity of hearts from normal and iron-deficient animals was approximately equal.

The authors point out that this pattern of different effects on the enzyme succinic dehydrogenase in different organs extends to the other iron enzymes studied by them. For example, cytochrome C was found to be depleted readily from rat liver and kidney, cytochrome oxidase only slightly from rat kidney, and catalase not at all from rat liver and red cells.

They caution, however, that the demonstration of diminished concentrations or activity of iron enzymes in certain organs cannot be considered evidence that deficiencies of these compounds are the cause of symp-

toms of iron deficiency any more than it can be concluded from the diminished hemoglobin concentration in the blood that lack of hemoglobin is the cause of symptoms in this disorder. They consider that elucidation of the relationship of iron-enzyme depletion and symptoms in the iron-deficiency state awaits development of means for the clinical measurement of iron enzymes in iron-deficient patients.

NECROTIZING CARDIOPATHY ASSOCIATED WITH CHLORIDE DEFICIENCY

Several drugs which produce cardiac necrosis in normal rats were found to be less toxic to rats fed a diet high in chlorides, and more toxic to rats fed a diet low in chlorides.

Although most of the once obscure forms of cardiac disease now have a rational explanation (S. Weiss and R. W. Wilkins, *Tr. Assn. Am. Phys.* **51,** *341 (1936)*, occasionally a patient is encountered who develops cardiac failure without apparent cause (R. J. Bing, *Circulation* **12,** *635 (1955)*). Nutritional deficiencies have been suspected in most such patients, but usually no specific response can be demonstrated by administering one or many of the vitamins.

Now attention is called to the role of the chloride ion in maintaining the integrity of cardiac function of animals (E. Bajusz and H. Selye, *Am. Heart J.* **60,** *266 (1960)*). The authors had previously studied a number of drugs or procedures which resulted in cardiac necrosis (Bajusz and Selye, *Tr. N. Y. Acad. Sci.* **21,** *659 (1959)*) and had noted that some of these were related to the concentration of electrolytes in the food.

The presently described study was designed by selecting 22 groups of rats with ten in each group. Half of the animals were given the usual laboratory diet which contained 0.935 per cent of chloride by weight. The other half of the animals ate the same diet but chlorides were restricted to 0.035 per cent. The first group of animals served as a control and each of the other groups received a drug or combination of drugs or some stressing procedure. By this experimental design they compared the effects of norepinephrine, vasopressin, plasmocid (an anti-malarial drug), papain, dihydrotachysterol plus calcium acetate, fluorocortisol plus disodium phosphate, fluorocortisol plus sodium acetate, fluorocortisol plus restraint, and fluorocortisol plus quadriplegia (induced by denervation of all four extremities).

In addition another series of 20 groups of rats (with ten in each group) was given a diet which would allow comparison of an increased amount of chloride. Half of these animals ate the regular diet and the other half ate a high chloride diet which contained 1.62 per cent of chloride by weight. These animals were exposed in a similar fashion to the same drugs but higher doses were used.

The results showed that norepinephrine and vasopressin had similar effects in normal animals and in those fed a low or high chloride diet. However, plasmocid, papain, dihydrotachysterol with or without sodium acetate, and fluorocortisol under all four situations, proved to be much more toxic and often was lethal to the rats fed a diet low in chlorides. Histologically the myocardium of these animals showed necrosis which varied with the type of toxic agent employed. The necrosis was extensive when papain or plasmocid had been given, and the vasoconstrictors caused patchy lesions, whereas dihydrotachysterol caused both necrosis and calcification of the myocardium. The addition of calcium acetate to the latter regimen increased the rate of calcification and resulted in calcification of the kidneys as well. In the group fed a low chloride diet and given fluorocortisol plus disodium phosphate, all of the animals died, whereas all of the controls in this group survived.

In the second part of this study which compared the effects of a high intake of

chloride, there was evidence of a considerable degree of protection against most of the forms of cardiac injury. Thus, there was prevention of calcium deposition in the heart as well as in the kidney when dihydrotachysterol and calcium acetate were administered. The lesions induced by papain and plasmocid were much less frequent and less severe in animals given a high intake of chloride. However, it was of particular interest that the animals given fluorocortisol and disodium phosphate plus the high chloride diet died promptly, presumably due to phosphate intoxication. No explanation was offered for this interpretation.

It was also noteworthy that the high chloride diet did not protect against the toxic effects of either vasopressin or norepinephrine.

This study calls particular attention to the necessity for an adequate quantity of chloride in the diet of animals in order to insure myocardial integrity and to protect against injurious agents. It would be foolish to attempt a conclusion regarding the value of chlorides in the treatment of human cardiac disease. However, these results are sufficiently suggestive to warrant investigation of the role that chlorides may play in the function of the human heart.

COPPER DEFICIENCY AND REPRODUCTIVE FAILURE

Copper deficiency results in a failure of reproduction which in the rat appears to be due to fetal death and resorption.

Low reproduction rates or reproductive failure of cattle are common complaints when copper is a limiting nutritional element. The pastures associated with poor reproductive performance may be low in copper or may contain normal amounts of copper that are poorly utilized because of other elements or compounds present.

The high incidence of reproductive failure in dairy cattle was emphasized by H. W Bennetts, A. B. Beck and R. Harley (*Aust. vet. J.* **24,** *237 (1948)*) in an Australian study of a copper-deficient area. Similarly, an English study of dairy cattle in peat soil pastures associated low blood copper values with poor reproduction. In the latter case the copper content of the pasture forage was normal but apparently unavailable because of other components (R. Allcroft and W. H. Parker, *Brit. J. Nutr.* **3,** *205 (1949)*).

Perhaps the low fertility is a reflection of other systemic changes; however, E. E. Van Koetsveld notes that lowered milk fat and lowered milk production, as well as achromotrichia, accompany the low reproductive rate in dairy cattle with lowered blood copper levels resulting from copper-deficient forage.

Although these and many other studies in different parts of the world have reported infertility in cattle and sheep as a result of a deficiency of copper in the diet, practically no systematic studies have been made of the relationship of copper in the diet to infertility in cattle or in laboratory animals.

The "swayback" syndrome in lambs dropped by ewes on a copper-deficient diet led B. Dutt and C. F. Mills (*J. Comp. Path. Therap.* **70,** *120 (1960)*) to attempt a study of the demyelination. They wished to produce a parallel condition of "swayback" in rats. However, female rats fed low-copper diets produced so few young that these investigators shifted their study to the relationship of copper level and reproduction.

In a preliminary study using a low copper diet (which gave good reproduction when supplemented with copper) Dutt and Mills found that only one out of 18 females produced a live litter and this rat immediately started to eat her young. Two other rats produced litters of dead pups.

Subsequently, four groups of three to four month-old female rats were placed on a diet containing from 0.35 to 0.45 p.p.m. of copper with supplements of 0, 0.5, 1.0 and 2 p.p.m. of copper and bred to normal males.

Interestingly enough, there appeared to be no cessation of estrus in the females on any of the four low copper levels as determined by vaginal smears made daily for a month before breeding. Subsequently, the rats were weighed daily until young were born.

Dutt and Mills observed that of the eight rats receiving an added 2 p.p.m. of copper, four produced litters ranging from three to seven pups. The young appeared normal. The daily weighing showed patterns of weight increase indicative of pregnancy in all the groups. Three patterns were followed: in the first there was a continuous increase in weight indicative of normal fetal growth; in the second, there was an increase in weight followed by a rapid decrease in live weight suggesting that conception occurred but was followed by fetal death and resorption or by abortion; and in the third there was a cyclical increase and decrease in live weight suggesting repeated conception, fetal growth, death, and resorption. With the exception of rats receiving the 2 p.p.m. added copper, all the females followed the second and third patterns.

Since no living young were produced at the lower levels of copper supplementation, Dutt and Mills decided to sacrifice all the animals for evidence of ovarian and uterine changes. Histological sections were made of uterine and fetal tissues and in all cases evidence suggested that the resorption of young was in progress or had occurred at every level of copper feeding.

The report of Dutt and Mills is an interesting contribution to the relation of copper and reproduction. The regular estrus differs from results with cattle where copper deficiency commonly causes early cessation of estrus. It is possible that the observation of Dutt and Mills reflects a prior nutritional status, since they placed their rats on the deficient diet and the next day began checking vaginal smears. This checking for estrus continued for a month when males were introduced into the cages with the experimental animals. It is likely that the copper reserves of the females were not depleted by that time.

It is interesting, however, that by one month after starting the experimental diets a copper deficiency caused a severe reproductive failure. The pattern of failure emphasizes that copper deficiency restricts the rapidly growing fetal tissues more severely than the more mature maternal tissues.

The reported symptoms of copper deficiency (which include nerve changes such as demyelination in "swayback" of lambs, achromotrichia in many species, diarrhea, lowered milk production, anemia and growth failure in cattle and sheep) all suggest a relationship of copper to rapidly metabolizing tissues. The report of Dutt and Mills extends these observations to the developing fetus of the rat. While limited in scope, this work is a "first step" toward a systematic study of the function of copper in reproduction and in the metabolism of rapidly growing tissues.

NOTES

Letter to the Editor

Dear Sir:

I was interested in the article on "Leaf Protein as Human Food" (*Nutrition Reviews* **18,** *218 (1960)*). I may refer in this connection to a note (B. Guha, *Lancet* **I,** *704 (1960)*) in which we mentioned that proteins from grass and water hyacinth were extracted by us during the famine in Bengal in 1943. These were fed to human beings without any untoward effects. More recently, we prepared a number of protein materials from green leaves of different species of plants on a fairly large scale and these were exhibited in the World Agriculture Fair in New Delhi held in December, 1959. These were also well received by visitors in the form of food preparations of the Indian type.

Our method can be used domestically. I believe, however, extraction on a large scale presents some practical difficulties, particularly in regard to a steady supply of the green leaves, which tend to putrefy in a tropical climate. Cold storage is expensive and would militate against the cheapness of the product.

B. C. Guha
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American Board of Nutrition Announcement

The American Board of Nutrition will hold the next examinations for certification as a Specialist in Human Nutrition on Monday, April 10, 1961, in Atlantic City. Candidates who wish to be considered for these examinations should forward applications to the Secretary's office not later than March 1. Application forms may be obtained from the Secretary, Robert E. Shank, M.D., Department of Preventive Medicine, Washington University School of Medicine, Euclid and Kingshighway, St. Louis, Missouri.

Blood Serum Proteins of the Hippopotamus

Opportunities to study the blood constituents of large wild animals in their native habitat are rarely presented to the biochemist so when an opportunity for such a study appears it is of interest.

In 1958 there were so many Hippopotamus amphibius (L) in the Kazinga Channel of Uganda's Queen Elizabeth National Park that they were overgrazing the vegetation and causing serious soil erosion problems. Efforts to drive the hippos to more suitable locations failed, so to keep their number in balance with the vegetation many had to be shot. Arrangements were made with various interested scientists to use this material.

B. M. Weinbren (*J. Comp. Path. Therap.* **70,** *217 (1960)*) secured blood samples from animals shot through the brain. Weinbren selected 100 non-hemolyzed samples for detailed study. The blood serum apparently was almost colorless, as Weinbren indicated that the serum of every species she had previously examined had had more pigment. This raises interesting speculation about the carotene absorption and conversion to vitamin A in this herbivorous species.

Of the 100 animals supplying blood for these studies 67 were adults, 43 males and 24 females. They showed considerable variation in serum proteins, although sex difference was hardly significant. The males showed averages for albumin 34.8 per cent, alpha globulin 7.7 per cent, beta globulin 16.5 per cent, gamma globulin 41.0 per cent, an albumin to globulin ratio of 0.54 and total serum protein of 9.4 g. per 100 ml. Comparable values for females were albumin 34.4 per cent, alpha globulin 7.3 per cent, beta globulin 17.8 per cent, gamma globulin 40.5 per cent. A/G ratio 0.53 and total serum protein 9.2 g. per 100 ml.

Values were obtained from seven young and 16 very old animals with slight difference

from the mean values obtained from the adult males and females. Interestingly enough, the total serum protein values are higher than those usually encountered in land animals; perhaps this value and the gamma globulin fraction which appears to account for the high total may be species specific. Perhaps because of the protein content the viscosity of the blood serum was high compared to sheep serum. Using water as a standard with a viscosity of 1.0, sheep serum viscosity was 1.6 and hippo serum 2.0.

Not often do large wild animals such as the hippopotamus become available for scientific study in numbers comparable to those reported in this study. The values obtained from the 100 animals selected provide a reliable norm for comparison with values obtained for single animals and for other species.

Recent Books

Races of Maize in Bolivia (*Publication 747*). Prepared by Richardo Ramirez E. *et al.* in collaboration with G. Edward Nicholson Calle, Edgar Anderson, and William L. Brown. Published by National Academy of Sciences-National Research Council, 2101 Constitution Avenue, Washington, D. C. Pp. 159. Price $1.50.

Food Becomes You. By Ruth M. Leverton. Published by Iowa State University Press, Ames, Iowa. Pp. 191. Price $3.50.

Review of Food Consumption Surveys. By Food and Agriculture Organization of the United States. July, 1958. Pp 107.

Food and Nutrition Services of Federal and Quasi-Official Agencies of the U. S. Agricultural Research Service of the United States Department of Agriculture, ARS 62-9. Washington, D. C. Pp. 45.

The Safe and Sure Way to Reduce. By Gaynor Maddox. Published by Random House, New York City, New York. Pp. 202. Price $3.95.

Pests, Pesticides and People. By Albert C. Worrell. Published by the American Forestry Association, 919 Seventeenth Street, N. W., Washington, D. C. Pp. 41.

The Nature and Fate of Chemicals, Applied to Soils, Plants and Animals. Published by United States Department of Agriculture, ARS 20-9, September, 1960. Pp. 221.

Calcification in Biological Systems. AAAS Symposium Vol 64. 1515 Massachusetts Avenue, N.W., Washington, D. C. Pp 526. Price $9.75. (AAAS members' cash orders $8.50).

Man and His Body. By Benjamin F. Miller and Ruth Goode. Published by Simon and Schuster, 630 Fifth Avenue, New York City. Pp. 361. Price $5.95.

Research Relating to Children, Bulletin 11, Clearinghouse for Research in Child Life, August 1959–January 1960. For sale by Supt. of Documents, U. S. Government Printing Office, Washington 25, D. C. Price $1.00.

Nutrition Animale. (Biologie, Physiologie et Alimentation Rationnelle) Vol. II by R. Jacquot *et al.* Published by J. B. Bailliere et Fils, Editeurs, 19 Rue Hautefeuille, Paris 4, France. (Vol. I Donnees Generals sur la Nutrition et L'Alimentation.) Both Vols. 963 pages.

The Memoirs of Ray Lyman Wilbur (1875–1949). By Edgar E. Robinson and Paul Carroll Edwards, Editors. Published by Stanford University Press, Stanford, California. Pp. 674. Price $10.00.

Endemic Goitre. (*World Health Organization, Geneva, Switzerland: Monograph Series, No. 44.*) By various authors, Geneva, 1960. Pp. 471. Price $8.00. £2, 24 Sw. fr. French and Spanish editions in preparation.

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The publication of NUTRITION REVIEWS has been undertaken to enable professionally trained people to keep abreast of current progress and to have available an unbiased, authoritative review of the current research literature in the science of nutrition.

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